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Shipboard test report

-Application for AMS determination-

Norwegian Institute for Water Research

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Shipboard testing of the KBAL® system of Knutsen Shipping OAS

Confidential Draft

Norwegian Institute for Water Research
– an institute in the Environmental Research Alliance of Norway

REPORT

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Abstract
The KBAL® system, developed by Knutsen Shipping OAS, was tested on board a ship according to the IMOs *Guidelines for approval of ballast water management systems (G8), Res. MEPC.174(58) Annex 4*. The tests were conducted on board the vessel m/t *Gijon Knutsen*, and a total of 5 test cycles were conducted to achieve 3 consecutive valid and successfull test cycles in compliance with the IMO requirements.

4 keywords, Norwegian	4 keywords, English
1. Ballastvann	1. Ballast water
2. Ombord	2. Shipboard
3. Testing	3. Testing
4. Knutsen Shipping OAS	4. Knutsen Shipping OAS

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Preface

The tests were conducted in 2011 and 2012 on board the vessel m/t *Gijon Knutsen.* NIVA conducted the testing as a contract assignment for Knutsen Shipping OAS, with Det Norske Veritas (DNV) as the verifying organisation.

During planning and conducting of the tests, Per Lother was the lead representative of Knutsen Shipping OAS, while Jad Mouawad has represented DNV in the project. From NIVA, August Tobiesen, Aina C. Wennberg and Stephanie Delacroix were the main representatives. Several other Knutsen Shipping OAS and NIVA staff have been involved in the project.

I will take the opportunity to thank Knutsen Shipping OAS for choosing NIVA as the main partner in the process of testing and verification of the KBAL® system, and thank all involved personnel for the stamina demonstrated in completing this project.

Oslo, April 2012

Stephanie Delacroix

Contents

Abbreviations and acronyms

BWMS – Ballast Water Management System
DNV – Det Norske Veritas
DO – dissolved oxygen
DOC – dissolved organic carbon
E. coli – Escherichia coli
GF/F – glass fiber filter grade F
GLP – Good laboratory Practice
IMO – International Maritime Organization
ISO – International Organisation for Standardization
KBAL – Knutsen's ballast water management system
n – number of measurements; in calculating the standard deviation
NIVA – Norwegian Institute for Water Research
NS-EN ISO – Norwegian, European and International Standard
OECD – Organisation for economic Co-operation and Development
POC – particulate organic carbon
PSU – Practical Salinity Unit (= ‰)
QAPP – quality assurance project plan
S1-S3 – sampling points 1-3
Std – standard deviation
TCB – thermotolerant coliform bacteria
TCBS – MacConkey and thiosulphate citrate bile salt agar
TSS – total suspended solids
X_i - individual analytical result; in calculating the standard deviation
X – the arithmetic mean of individual analytical results; in calculating the standard deviation

Summary

Shipboard testing of the KBAL system of Knutsen Shipping OAS was completed in the period of June 2011 to March 2012, a period exceeding 6 months, onboard the vessel *m/t Gigon Knutsen* along the Denmark and Holland coasts. The testing was conducted according to the IMOs *Guidelines for approval of ballast water management systems (G8), MEPC 58/23/ Annex 4, Res. MEPC 174/58* (IMO, 2008), hereafter referred to as G8. A total of 5 test cycles were completed (Table 1). Regulation D-2 of the IMO convention and the required biological water quality in uptake ballast water, in treated ballast water, and in control ballast water on discharge, as stated in regulation G8, is given in Table 2.

Fulfilment of the biological requirements of the uptake ballast water

- The required density of the ≥50 µm group (viable organism concentration exceeding 10 times the values of Regulation D-2.1) was met in all tests cycles (**Table 16**).
- The requirement in the ≥10-50 µm group (viable organism concentration exceeding 10 times the values of Regulation D-2.1) was met in the 3 last test cycles only (**Table 17**).

Biocidal effects of treatment

- The required "less than 10 viable organisms ≥50 µm in minimum diameter per m^3 in the treated water" was fulfilled immediately after discharge only in test cycle 5, and in test cycles 3, 4 and 5 24h after discharge. The equivalent requirement of the non-treated control water, stating that the level of viable organisms at deballasting should be higher than 10 per m^3, was fulfilled in all test cycles (**Table 16**).
- The required "less than 10 viable organisms ≥10 - 50 µm in minimum diameter per ml in treated water on deballasting", was reached in all test cycles. The equivalent requirement for the non-treated control water upon deballasting states that the level of viable organisms should be higher than 10 per ml. This requirement was fulfilled in all test cycles (**Table 17**).
- Regulation D-2 requires the maximum concentrations of *Escherichia coli*, *Vibrio cholerae* (toxicogenic serotypes O1 and O139) and Intestinal *Enterococci to be*; <250 cfu/100ml, <1 cfu/100ml and <100 cfu/100ml, respectively, in the treated ballast water upon discharge. These requirements were fulfilled for all the bacteria in all test cycles (**Table 19**).

An overview of test cycles in compliance with IMO requirements, and not in compliance with the requirements, is shown in **Table 1**. The three last consecutive test cycles were both valid and successful tests according to G8 requirements.

Operational performance of KBAL system

As specified in chapter 1.3.2.3 of the QAPP, the Sea KBAL system was designed for treatment of 600 m^3/h water supplied by both ship's pump and the KBAL booster pump. The pressure before the reactor shall be minimum 4.5 bara and 3.5 barg, where Barg is Gauge Pressure and Bara is Absolute Pressure. Pressure after the reactor shall be 0.012 to 0.03 bara (-0.988 to -0,970 barg). The UV unit should give an UV dose of minimum 60mJ/cm^2 according to land-based testing results (see NIVA's report No.6164-2011).

- The average flow rate variation was from 598-663 m^3/h for all test cycles and all water transfers (**Table 14**).
- The average UV dose was above 100mJ/cm^2 during both ballasting and deballasting for the three last test cycles. The UV dosage measurements could not be recorded for the two first test cycles because the UV sensor was defect (**Table 14**).
- The pressures before the reactor and after reactor were higher than 3.5 barg and lower than -0.970 barg respectively for all test cycles and water transfers. The low standard deviations of the pressures measurements indicate the stability of the system over the whole water transfer for all test cycles (**Table 14**).

All test cycles were performed according to the operation manual of the KBAL system.

Table 1. Overview of the results from Test cycles 1-5, including concentrations of viable organisms in the different size classes in compliance (green background) or not in compliance (red background) with the IMO requirements for the different uptake and discharge ballast waters. Viable organisms in the ≥10-50 µm size class were detected by the CFDA-methodology. The results are reported as mean value of triplicate samples for control water and treated water during ballasting and of 9 samples for treated water during discharge, including standard deviations. The results for ≥50 µm organism are given both immediately after discharge and 24h after sampling. The 24h after discharge treatment results indicated in this table were corrected by the mean mortality of the organism in discharge control samples during the 24h storage after discharge sampling, to take into account the mortality due to the 24h storage sampling procedure and not to the KBAL treatment.

Test cycle	Uptake water for the ballast water to be treated		Uptake water for the control tank		Treated ballast water on discharge			Control ballast water on discharge	
	≥50 µm	≥10-50 µm	≥50 µm	≥10-50 µm	≥50 µm	≥50 µm 24h later	≥10-50 µm	≥50 µm	≥10-50 µm
Requirement	>100	>90	>100	>90	<10	<10	<10	>10	>10
1	43 278 ± 4 945	66 ± 16	27 359 ± 6 751	53 ± 10	27 ± 32	23	<1	18 421 ± 377	43 ± 9
2	5 505 ± 670	76± 6	5 120 ± 588	71 ± 14	77 ± 23	-	<1	4 382 ± 1 270	35 ± 8
3	181 625 ± 8 205	311 ± 118	172 773 ± 94 788	508 ± 32	12.8 ± 9.8	2.6	<1	203 338 ± 96566	520 ± 100
4	147 813 ± 29 030	508 ± 230	128 833 ± 70 172	256 ± 36	10.7 ± 12.1	2.4	<1	100 033 ± 12 266	475 ± 120
5	22 569 ± 5 181	259 ± 107	22 822 ± 5 642	416 ± 156	0.6± 1.3	0.6	3.2 ± 2.7	8 834 ± 5 391	431 ± 204

Table 2. Regulation D-2 of the IMO convention and the required biological water quality in uptake ballast water, in treated ballast water, and in control ballast water on discharge, as stated in regulation G8 by IMO.

Regulation D-2 Ballast Water Performance Standard			
1	Ships conducting Ballast Water Management in accordance with this regulation shall discharge less than 10 viable organisms per cubic metre greater than or equal to 50 micrometres in minimum dimension and less than 10 viable organisms per milliliter less than 50 micrometres in minimum dimension and greater than or equal to 10 micrometres in minimum dimension; and discharge of the indicator microbes shall not exceed the specified concentrations described in paragraph 2.		
2	Indicator microbes, as a human health standard, shall include: .1 Toxicogenic *Vibrio cholerae* (O1 and O139) with less than 1 colony forming unit (cfu) per 100 milliliters or less than 1 cfu per 1 gram (wet weight) zooplankton samples ; .2 *Escherichia coli* less than 250 cfu per 100 milliliters; .3 Intestinal Enterococci less than 100 cfu per 100 milliliters.		
Biological requirements for shipboard testing			
Organism group	**Uptake water for both the control tank and the ballast water to be treated**	**In treated ballast water on discharge**	**In control ballast water on discharge**
≥50 µm min. dimension	Viable organism concentration exceeding 10 times the values of Regulation D-2.1 Concentration that must be achieved: >100 viable organisms per m^3	In compliance with Regulation D-2 Concentration that must be achieved: <10 viable organisms per m^3	Viable organism concentration exceeding the values of Regulation D-2.1 Concentration that must be achieved: >10 viable organisms per m^3
≥10-50 µm min. dimension	Viable organism concentration exceeding 10 times the values of Regulation D-2.1 Concentration that must be achieved: >90 viable organisms per ml	In compliance with Regulation D-2 Concentration that must be achieved: <10 viable organisms per ml	Viable organism concentration exceeding the values of Regulation D-2.1 Concentration that must be achieved: >10 viable organisms per ml
Vibrio cholerae	-	<1 cfu/100 ml	-
Escherichia coli	-	<250 cfu/100 ml	-
Intestinal *Enterococci* (Serotype O1 and O139)	-	<100 cfu/100 ml	-

There are no requirements regarding chemical water quality. Uptake water was analysed with respect to temperature, salinity, particulate organic carbon and total suspended solids as required. The analyses show that a wide variety of water qualities were covered by the conducted tests, with variations in temperature from approximately 6 to 22 ºC, in salinity from 28 to 31 PSU, in particulate organic carbon from 0.2 to 0.7 mg/l, and suspended solids from 2 to 12 mg/l.

1. Background

The overall goal for Knutsen Shipping OAS is to acquire type approval certification of the KBAL system in accordance with the requirements in the IMO Convention on ballast water management and underlying guidelines, including the *Guidelines for approval of ballast water management systems (G8), Res. MEPC.74(58) Annex 4*, hereafter referred to as G8.

Land-based pilot tests were conducted by NIVA in the period of September 2010 to January 2011 for partial fulfilment of such a certification. The objective of the land based testing was to verify the biological performance characteristics of the KBAL system technology relative to the biological requirements stated in the IMO G8 Guidelines. The results were reported in the NIVA's report No. 6164-2011.

The experience from the land-based testing served as a fundament for shipboard testing of the technology. Shipboard testing for type approval, as reported here, was conducted in the period of June 2011 to March 2012. The tests were conducted in accordance with G8.

2. Infrastructure and test protocols

2.1 Description of ship and technology set-up

2.1.1 Ship and shipboard installation

The shipboard testing of the KBAL system of Knutsen Shipping OAS was conducted on board of the vessel m/t *Gijon Knutsen* (**Figure 1**).

m/t Gijon Knutsen

GIJON KNUTSEN

FLAG	UNITED KINGDOM
PORT OF REGISTRY	ABERDEEN
CALL SIGN	MMLG6
IMO NO.	9313527
SHIP COMMUNICATIONS	TELEPHONE +47 52865837, +47 52865838 +870 600828164, +870 764626696 FAX +47 52865839, +870 764626697 TELEX 423 500 984, 423 500 985 MOBILE +47 90826130 E-MAIL GIJON.KNUTSEN@KNUTSENOAS.COM
OWNER	KNUTSEN PRODUCT TANKER X 5504 HAUGESUND NORWAY
MANAGING OWNER	KNUTSEN OAS (UK) LTD. 2 QUEENS CROSS ABERDEEN AB15 4YB SCOTLAND TELEPHONE +44 1224 618 420 FAX +44 1224 624 891 E-MAIL: TECHNICAL.ABERDEEN@KNUTSENOAS.COM
BUILDER	NAVAL GIJON, SPAIN
BUILT	JUNE 2006
CONVERTED	REMONTOWA S.A. GDANSK, POLAND - JULY 2006
DESCRIPTION	THE VESSEL I S A SHUTTLE TANKER WITH A BOW LOADING SYSTEM AND A DYNAMIC POSITIONING SYSTEM. 14 CARGO TANKS.
MAIN D IMENSIONS	LENGTH OVER ALL 186,93 M LENGTH BETWEEN PP 174,00 M BREADTH MOULDED 27,40 M DEPTH MOULDED 16,90 M DRAUGHT ON SUMMER FREEBOARD 11,50 M DEADWEIGHT SUMMER DRAFT 35.168 MT

TONNAGE	**DESCRIPTION**	**INTERNATIONAL**	**SUEZ**	**PANAMA**
	GROSS (GT)	24242	24812,27	
	NET (NT)	10125	20611,42	20182

CLASS: DET NORSKE VERITAS
✠1A1 TANKER FOR OIL, PRODUCTS AND CHEMICALS, ESP, E0, COW, BOW LOADING, DYNPOS AUTR

Figure 1. Information on the vessel *m/t Gijon Knutsen.*

Configuration of the test tanks with pipes, valves and devices is shown in **Figure** .

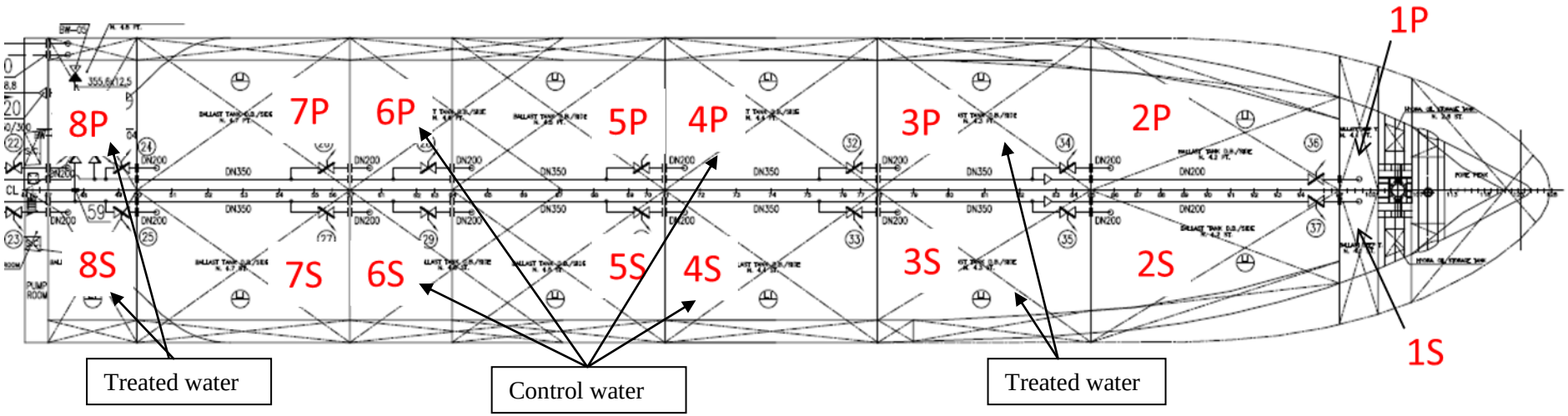

Figure 2a. Configuration of the ballast tanks on board of the ship.

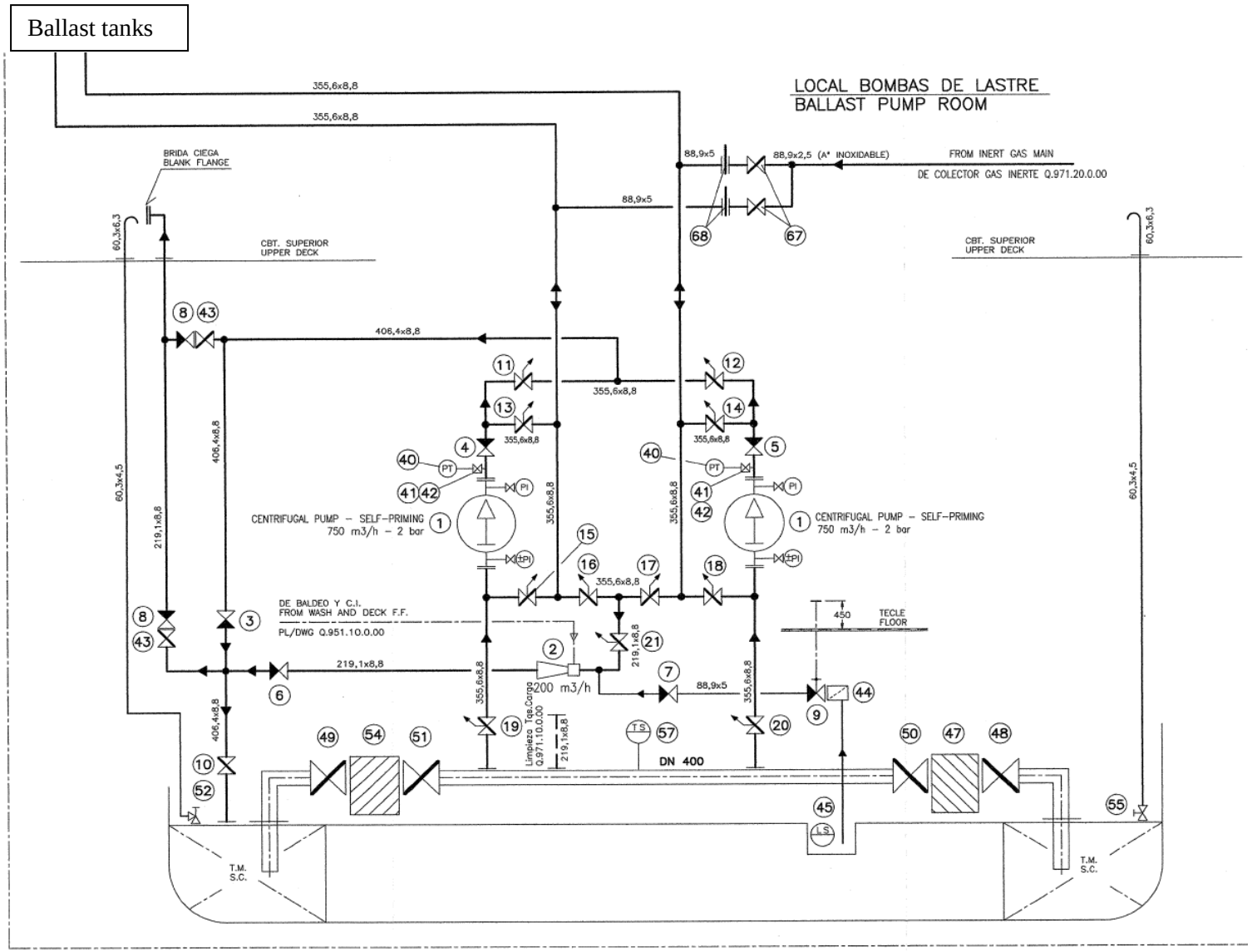

Figure 2b. Configuration of the pipelines from seachest, through KBAL system and to the ballast water tanks.

The tanks used for testing have the following labelling presented in **Table 3**. Because of the pipelines configuration of the ship, all treated water transfers during ballasting were performed through the tank no. 8 portside (with a volume capacity of 536m^3) before storage in the tanks as presented in the **Table 3**. To illustrate this, during ballasting operation of the test cycles 1 and 2, the seawater was pumped from the seachest to the KBAL sytem and through the tank 8P on its way to the tanks 3S and 3P.

Table 3 Overview of the ballast tanks used for testing and their water volume capacity for both treated and control water. (s: starboardside, p: portside)

	Tank. identification	**Tank's volume capacity (m^3)**	**Test cycles no.**
Treated water tanks	8p, 3s and 3p	1166 and 1117	1,2
	8s and 8p	536 and 492	3,4,5
Control water tanks	6s and 6p	1085 and 1197	1
	8s	536	2
	6p	1197	3,4
	4s and 4p	1228 and 1081	5

2.1.2 The KBAL water management system

Process description

Ballast water was treated during both ballast intake and during discharge by both the reactor unit and the UV unit. Treatment at discharge was done to eliminate re-growth and insufficient intake disinfections. A process description with the start and stop procedures during ballasting and discharge after treatment by the KBAL system is found in"Appendix G – Process description".

System and component description

The KBAL system of Knutsen Shipping OAS is a complete ballast water treatment system composed of two main components (**Figure 3**); a reactor unit that damages the larger organisms by pressure drop, 2) and a cross flow UV unit with 12 medium pressure UV lamps to disinfect and inactivate smaller plankton, bacteria and viruses.

KBAL system includes a control and auxiliary equipment including sampling points to control water flows and measure different alarm levels during operation, and to collect samples during operation and testing.

Due to the pressure required at the inlet to the reactor, a booster pump is installed since the ship's pumps did not have sufficient head for the KBAL system.

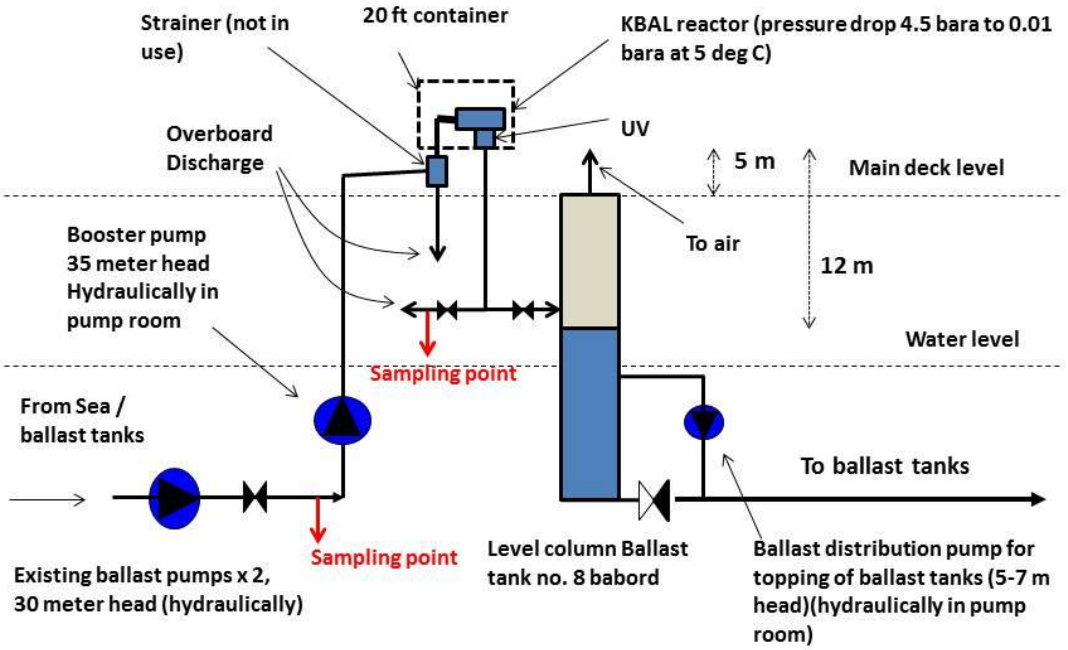

Figure 3. KBAL system and composition.

Physical properties of the KBAL test equipment

The KBAL System was installed in a container on deck on board of m/t *Gijon Knutsen (***Figure 4**). The test equipment was tested at a max flow rate of 600 m^3/h. The system has one reactor unit and one UV unit consisting of twelve UV lamps. The two pumps on board (for portside and babord side) were not sufficient for the inlet pressure of KBAL, therefore a booster pump was installed (**Figure 3**).

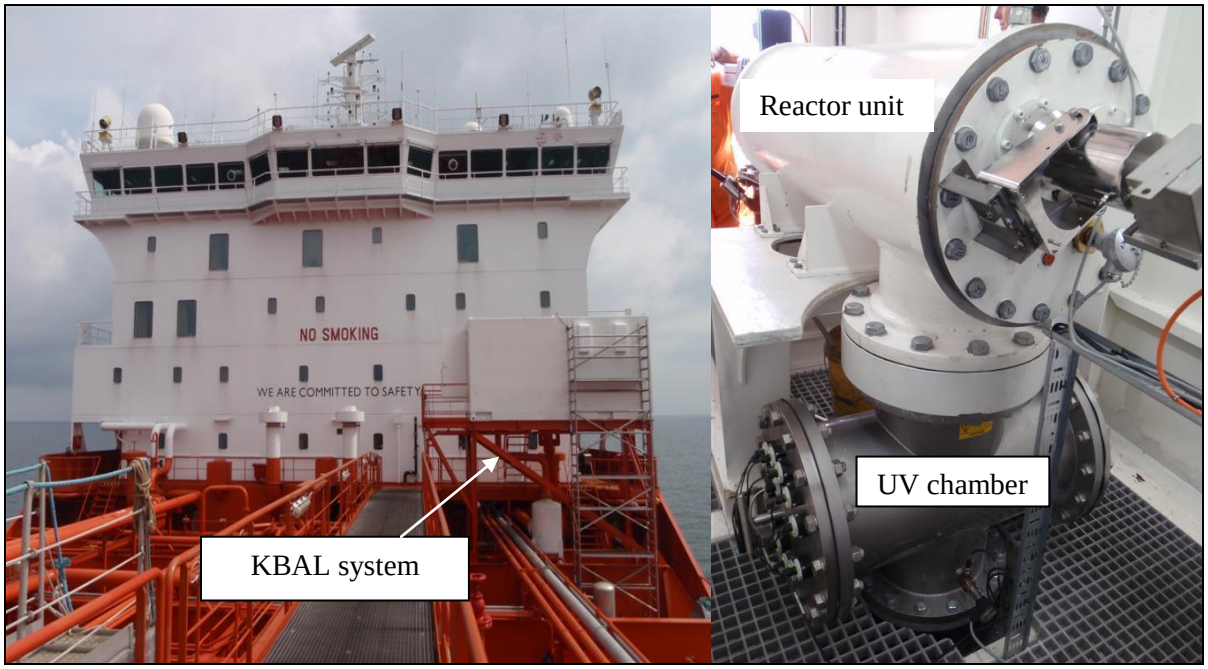

Figure 4. The KBAL System in a container over deck on board of m/t *Gijon Knutsen*.

2.1.3 Overview of water quality requirements of shipboard testing

Table 4 summarizes the biological requirements regarding biological water quality in uptake ballast water, in treated ballast water, and in control ballast water on discharge, as stated in regulation G8 by IMO.

Table 4. Regulation D-2 of the IMO conventionan and the required biological water quality in uptake ballast water, in treated ballast water, and in control ballast water on discharge, as stated in regulation G8 by IMO.

Regulation D-2 Ballast Water Performance Standard			
1	Ships conducting Ballast Water Management in accordance with this regulation shall discharge less than 10 viable organisms per cubic metre greater than or equal to 50 micrometres in minimum dimension and less than 10 viable organisms per milliliter less than 50 micrometres in minimum dimension and greater than or equal to 10 micrometres in minimum dimension; and discharge of the indicator microbes shall not exceed the specified concentrations described in paragraph 2.		
2	Indicator microbes, as a human health standard, shall include: .1 Toxicogenic *Vibrio cholerae* (O1 and O139) with less than 1 colony forming unit (cfu) per 100 milliliters or less than 1 cfu per 1 gram (wet weight) zooplankton samples ; .2 *Escherichia coli* less than 250 cfu per 100 milliliters; .3 Intestinal Enterococci less than 100 cfu per 100 milliliters.		
Biological requirements for shipboard testing			
Organism group	**Uptake water for both the control tank and the ballast water to be treated**	**In treated ballast water on discharge**	**In control ballast water on discharge**
≥50 µm min. dimension	Viable organism concentration exceeding 10 times the values of Regulation D-2.1 Concentration that must be achieved: >100 viable organisms per m^3	In compliance with Regulation D-2 Concentration that must be achieved: <10 viable organisms per m^3	Viable organism concentration exceeding the values of Regulation D-2.1 Concentration that must be achieved: >10 viable organisms per m^3
≥10-50 µm min. dimension	Viable organism concentration exceeding 10 times the values of Regulation D-2.1 Concentration that must be achieved: >90 viable organisms per ml	In compliance with Regulation D-2 Concentration that must be achieved: <10 viable organisms per ml	Viable organism concentration exceeding the values of Regulation D-2.1 Concentration that must be achieved: >10 viable organisms per ml
Vibrio cholerae	-	<1 cfu/100 ml	-
Escherichia coli	-	<250 cfu/100 ml	-
Intestinal *Enterococci* (Serotype O1 and O139)	-	<100 cfu/100 ml	-

There are no requirements regarding chemical water quality for shipboard testing. Uptake water should be analysed with respect to temperature, salinity, particulate organic carbon and total suspended solids.

2.2 Running test cycles

The different water transfers between tanks via the KBAL system during a test cycle is shown in **Figure 5**. One test cycle involves consecutive treatment of test water during ballasting by the reactor unit and the UV unit of the KBAL system transferring the test water from the sea to a tank for treated water (**Figure 5a)**. A second treatment of the treated water is conducted by both the reactor unit and the UV unit during deballasting (**Figure 5b).**

A control cycle (**Figure 5c)** is run by ballasting seawater to a tank for control water using the ballast pump of the ship, but in by-pass of the treatment units of the KBAL system. The deballasting of control water is also conducted by pumping only, without any treatment (**Figure 5d)**.

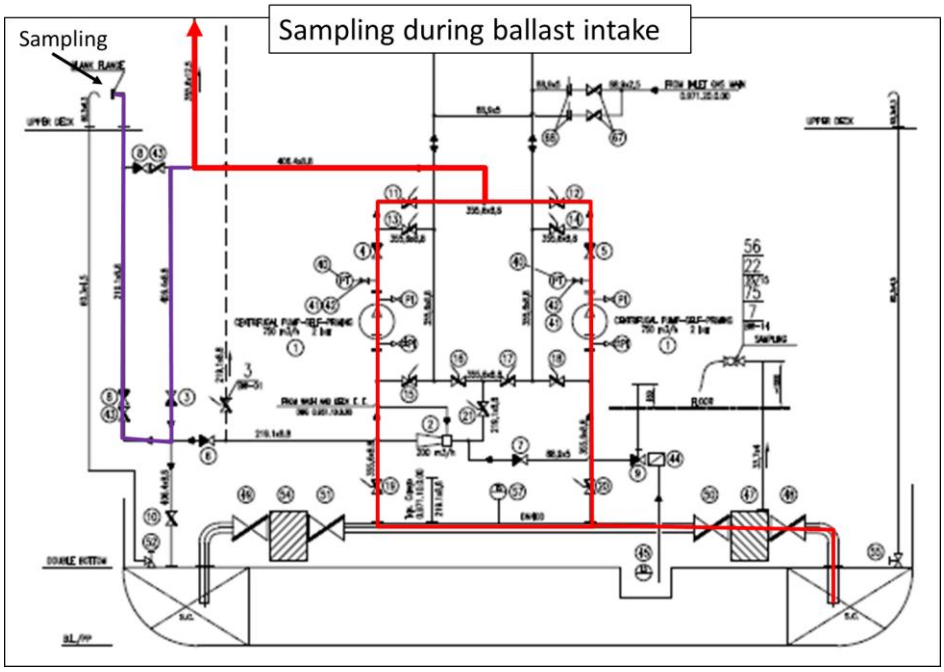

Figure 5a. Ballasting of the treated water.

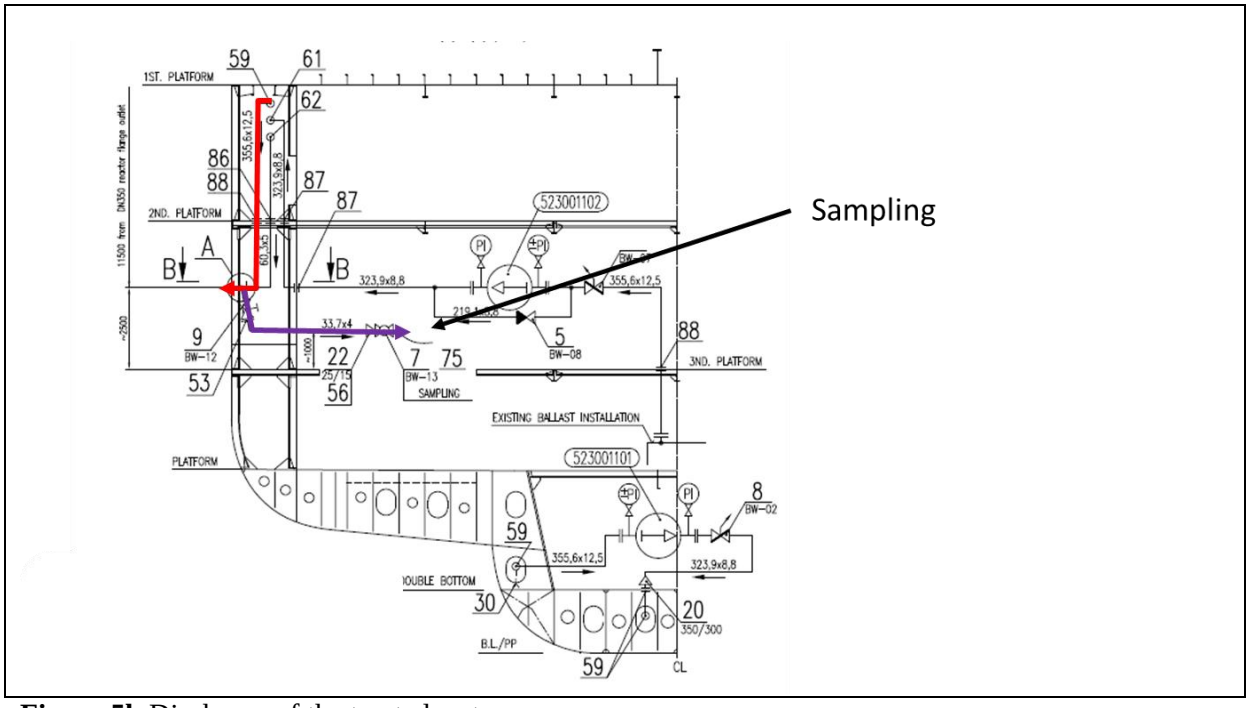

Figure 5b Discharge of the treated water.

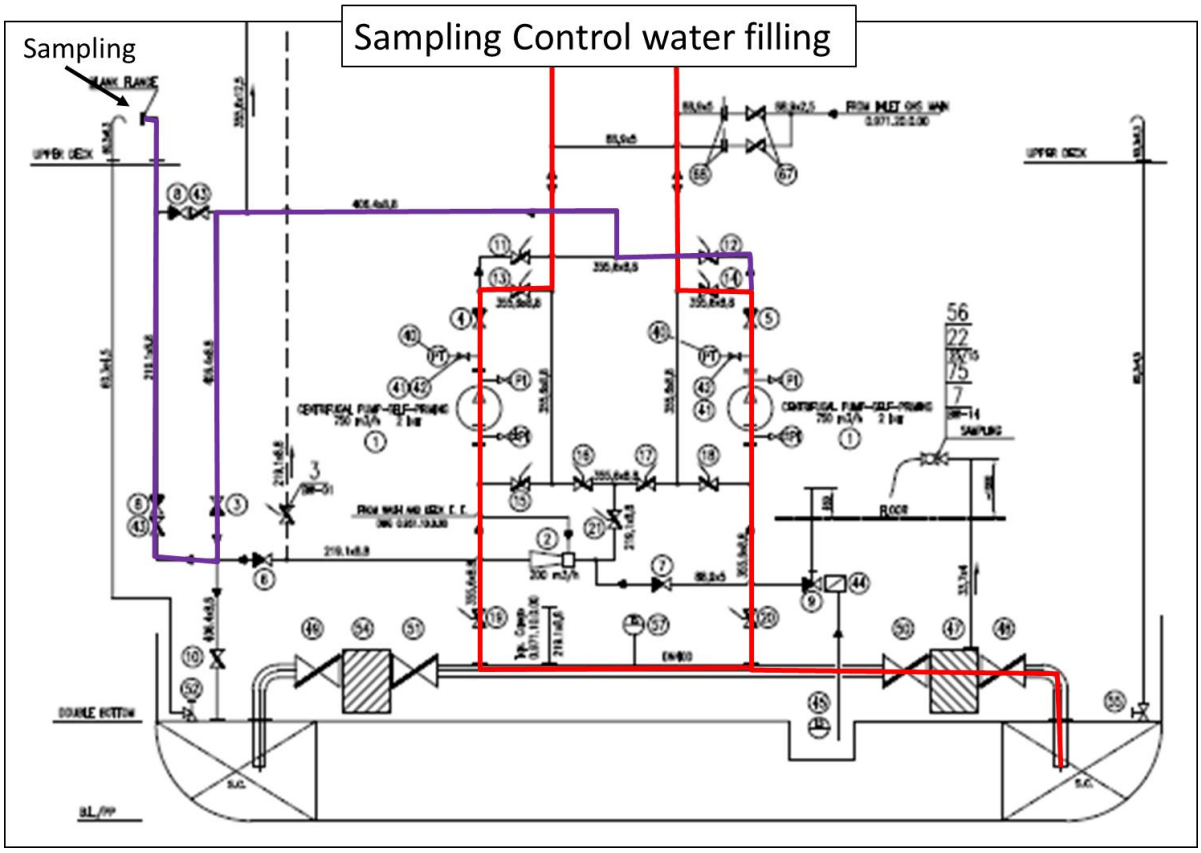

Figure 5c. Ballasting of the control water.

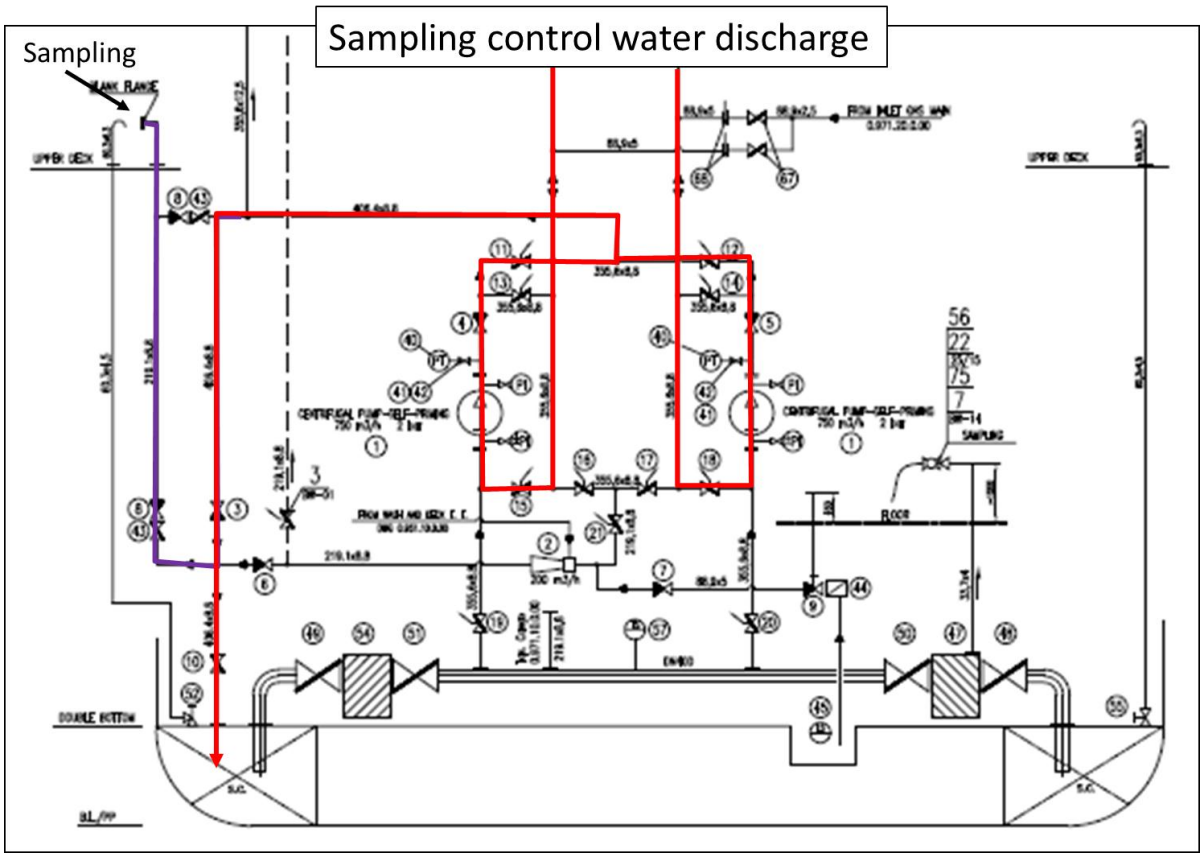

Figure 5d. Discharge of the control water.

2.3 Sampling

The different sampling points at different water transfers (**Table 5**) between tanks when applicable via the KBAL system during a test cycle including the control is shown in **Figure 5**.

The influent water to the control tank is sampled (S1) from the sea chest (valve Q1) simultaneously with ballasting of the tank for control water (Figure 5c). The influent water to the KBAL treatment process is sampled (S2) from the sea chest (valve Q1) simultaneously with ballasting (and treatment of the water) of the tank for treated water (Figure 5a).

The discharge treated water is sampled (S3) from valve Q2 simultaneously with deballasting (and deballast treatment) of the tank for treated water (Figure 5b). The discharge control water is sampled (S4) from valve Q1 upon deballasting of the tank for control water (Figure 5d). Valves Q1 and Q2 are equipped with sampling tubes described in Appendix H of the QAPP.

Table 5 Overview of sample collection.

Sample description	Abbreviation	Sampling point	Number of replicates
Influent water to control tank	S1	Seachest, valve Q1	3*
Influent water to treated tank	S2	Seachest, valve Q1	3*
Discharge treated water	S3	Valve Q2	9**
Discharge control water	S4	Valve Q1	3*

*Three replicate samples to be collected over the period of uptake (e.g. beginning, middle, end)
**Three replicate samples to be collected at each of three times during the period of discharge (e.g. 3x beginning, middle, end).

Samples were collected to document:
1) the source water chemical characteristics as defined by IMO,
2) that the water quality in the influent seawater is meeting the biological water quality criteria defined by IMO,
3) that the water quality in the discharge control water is meeting the biological water quality criteria defined by IMO,
4) that the water quality in the discharge treated water is meeting the biological water quality criteria defined by IMO,
5) the efficiency of KBAL system in removing/inactivating target organisms in the ballast water upon discharge
6) the water quality in the control tank upon discharge .

Procedures for sampling and water transfers are described in detail in section 3.

Figure 6. Arrangement of flowmeter for measuring volume of samples and collection of samples for analysis of organisms ≥50 µm during ballasting of both treated and control waters.

2.4 Sample analysis

Table 6 summarizes all types of measurements taken during the study.

Table 6. Parameters measured during the study; type of sampling and measurement location.

Parameter	Sample number	Type of sample	Location for analysis
Operational parameters for the KBAL system			
Flow	S1, S2, S3, S4	Continuous	On board Gijon
UV dosage	S1, S2, S3, S4	Continous	On board Gijon
Pressures before and after reactor	S1, S2, S3, S4	Continous	On board Gijon
Chemical water quality measurements			
Temperature	S1, S2, S3, S4	*In situ*, continuous	On board Gijon
Salinity	S1, S2, S3, S4	Discrete grab	Gijon and NIVA
UV transmission (UV-T)	S1, S2, S3, S4	Discrete grab	Gijon and NIVA
Particulate organic carbon (POC)	S1, S2, S3, S4	Discrete grab	NIVA laboratory
Total suspended solids (TSS)	S1, S2, S3, S4	Discrete grab	NIVA laboratory
Biological treatment performance parameters			
Organisms ≥50 µm	S1, S2, S3, S4	Discrete grab	On board Gijon
Organisms ≥10-50 µm	S1, S2, S3, S4	Discrete grab	Gijon and NIVA
E. coli	S1, S2, S3, S4	Discrete grab	Gijon and NIVA
Intestinal *Enterococci*	S1, S2, S3, S4	Discrete grab	Gijon and NIVA
Vibrio sp.	S1, S2, S3, S4	Discrete grab	Gijon and NIVA
Vibrio cholerae (Serotype O1 and O139	S1, S2, S3, S4	Discrete grab	NIVA laboratory

3. Sampling procedures

3.1 Representativeness of samples

Separate sampling lines from the valves to the sample points were used to assure that representative samples were drawn. Pipes were flushed with a volume corresponding to >1 times the piping volume (3 minutes) prior to sampling.

3.2 Sampling of test waters

Table 7 summarizes the sampling equipment used to collect samples for the individual parameters.

Table 7. Equipment and containers used for sampling and necessary and sampled volume for the individual parameters.

Parameter	**Sampling equipment**	**Sample container**	**Collected volume S1, S2, S3, S4**
Temperature	Temp. meter	-	-
Salinity	Probe	-	-
POC	Directly	Clean plastic bottle	1000 ml
TSS			
UV-Transmission			
Organisms ≥ 50µm	Sieving*	Clean glass bottle	1 m^3
Organisms ≥10-50µm	Directly	Clean glass bottle	1000 ml
E. Coli	Directly	Sterile Plastic bottle with thiosulfate	2x500ml
Intestinal *Enterococci*			
Vibrio cholerae			

* A 1 m^3 sample is concentrated to a volume of 40-100 ml through a plankton net with diagonal dimensions of 50 µm.

The procedures for collecting samples from sampling lines from the different sampling valves are as follows:

1) Sampling of organisms ≥ 50 µm (S1, S2, S3 and S4): A plankton net is placed in a plastic container and is used to collect a 1 m^3 sample. The sampled water is slowly sieved through a plankton net (50 µm diagonal dimensions) attached to a plastic cup, collecting the organisms in the cup. The volume of the sample is determined by measuring the volume passing out of the plastic container by a Siemens DN-50 flowmeter.

2) Sampling of organisms≥ 10-50 µm (S1, S2, S3 and S4): Organisms with a minimum diameter between 10 µm and 50 µm are sampled in 1000 ml bottles.

3) Sampling of bacteria (S1, S2, S3 and S4): Bacterial samples are sampled in 2x 500ml sterile bottles with thiosulfate.

4) Sampling for salinity, POC, TSS and UV-Transmission analysis (S1, S2, S3 and S4): samples are collected in 1x 1000ml clean plast bottles.

3.3 Sample preservation

Preservation methods and expected storage/holding times before measurement are shown in **Table 8**.

Samples for ≥50µm organism were analysed immediately after sampling on board the ship. Samples for re-counting after 24h storage, were stored in a dark and cooling room (4 ^{o}C).

Samples for ≥10-50µm organism were stored in a cooling room (4 ^{o}C) on board the ship between 24h and 72h, depending of the voyage period, before to be transported off the ship to the NIVA's laboratory for analysis.

Samples for bacteriological analysis were either stored on board in a cooling room (4 ^{o}C) and transported off the ship to be analysed within the standard timeframe at NIVA's laboratory, or the samples were filtered and incubated on-board. If the incubated plates were taken from the ship prior to completion of the incubation period, the incubation period on the ship was extended as long as possible, and the incubation was continued as soon as possible at NIVA's laboratory.

Samples for chemistry analysis (TSS) were either transported off the ship to be analysed within the standard timeframe at NIVA's laboratory, or the samples were filtred, and both samples and filters were stored in cooling room on board before transported off to the NIVA's laboratory to be continued analysed.

Table 8. Preservation methods and expected storage/holding times before measurement (ISO/CD 5667-3, 2001).

Parameter	**Preservation**	**Note**	**Maximum holding time**	**Expected storage time**
Temperature	-	On board	-	-
Salinity	4^{o}C and dark	On board NIVA/local lab	7 days	≤2 days
Particulate organic carbon (POC)	4^{o}C and dark	NIVA/local lab	7 days	≤7 days
Total suspended solids (TSS)	Filtration, 4^{o}C and dark	on board NIVA/local lab	24 hours	≤24 hours
UV Transmission (UV-T)	4^{o}C and dark	on board NIVA/local lab	48 hours	≤48 hours
Organisms ≥ 50 µm (immediately after sampling)	4^{o}C and dark	On board	6 hours	≤ 2 h
Organisms ≥ 50 µm (24h after discharge sampling)	1:25 dilution of the control samples 4^{o}C and dark	On board/NIVA lab	24 hours	24 hours
Organisms ≥10-50 µm	4^{o}C and dark	On board/NIVA lab	72 hours	≤24 hours
E. Coli	4^{o}C and dark	On board/NIVA lab/local lab	24 hours	≤ 24 hours
Intestinal *Enterococci*				
Vibrio cholerae				

3.4 Sample transportation

For samples to be transported and analysed off-board the following applies. All preserved samples were stored on board in a cooling room (4 ^{o}C) and transported to NIVA as luggage in a cooler package with frozen cooling pack (0-5 ^{o}C).

3.5 Measures to avoid cross-contamination during test water transfer and sampling

To avoid cross-contamination between consecutive test waters upon transfer in the ballast system, all sampling pipes were flushed to ensure that the entire water volume in the pipes was replaced with treated water before each treated water sampling.

The volume of the ballast piping system was flushed out during the warming up of the UV system as described in the start-up procedures in chapter 2.1.2 of the QAPP.

Plankton nets were dedicated to either treated or not treated sample water. Plankton nets were rinsed between samples. All plankton sample bottles were thoroughly cleaned before use in sea water between each sampling.

4. Testing and measurement protocols

An overview of all analytical measurements with instruments used, references and current uncertainty of the method applied is described in **Table 9** (chemical) and **Table 10** (biological).

4.1 Biological measurements – initial quick tests

Rapid tests were performed to verify the presence of sufficient organisms according to point 2.2.2.5 in the G8 Guideline: "Valid tests are indicated by uptake water, for both the control tank and ballast water to be treated, with viable organism concentration exceeding 10 times the values of Regulation D-2.1. This would require that there is >100 organisms/ml of size ≥10-50 µm and >100 organisms/m^3 of >50 µm. Initial rapid tests are performed until the required organism concentration is achieved.

4.1.1 Rapid method of evaluation of density of ≥10-50 µm organisms

Take out 100 µl and view at 40x magnification and count cells larger than 10 µm in minimum dimension. Target count is >10 cells in 100 µl in sample (target value >100 cells/ml).

4.1.2 Rapid method of evaluation of density of ≥50 µm organisms

Sieve 100 L seawater sample from sea chest through 50 µm Nitex screen. Resuspend in 10-50 ml. View sample in field microscope at 10x magnification counting organisms ≥ 50 µm in minimum dimension. Sample should contain >10 organisms (target value > 100 organisms/m3).

4.2 In situ measurements

4.3.1 Salinity

Salinity was measured using a calibrated probe on board and at the NIVA laboratory.

4.3.2 UV transmission (UV-T)

UV-transmission (UV-T) was measured using a spectrophotometer method on board or/and at the NIVA laboratory using a calibrated spectrophotometer. The absorbance of the unfiltered sample is measured in a 1cm quartz cuvette against distilled water at a wavelength of 253.7 nm. The results are reported in percentage (%).

4.3.3 Particulate organic carbon (POC)

POC was measured at NIVA (method G6) as the amount of organic matter accumulating on a glass fibre filter GF/F (0.7 µm) when a known amount of sample is filtered. The dry sample is encapsulated in tin capsules which are ignited in oxygen saturated heliumgas at 1800 ºC. Surplus oxygen is removed by Cu at 650 ºC and the off-gases are passed through a chromatographic column, where upon CO_2 is detected (Thermo Flash 2000 element analyzer). The method is based on CARLO ERBA ISTRUMENTAZIONE, ELEMENTAL ANALYZER 1106. Instruction manual, APPLICATION LAB REPORTS, Elemental analysis lab, Carlo Erba. January 1987. Detection limit is depending of the volume of sample filtered. For 50-100ml filtered sample, the detection limit is between 0.05 and 0.1 mg C/l.

4.3.4 Total suspended solids (TSS)

TSS was measured at NIVA (method B1/2) in accordance to NS-EN 872 and NS 4733. A glassfiber filter GF/F (0.7 µm) is washed with distilled water, dried at 105^{o}C for 30 minutes, then ignited at 480^{o}C for 2 hours and finally weighed. The sample is filtered through a filter prepared as described earlier. The filtered samples are dried for 1 hour and weighed. The TSS is represented by the weight increase. Lowest reported value: 0.1 mg/l.

4.3.5 Organisms ≥50 μm

Organisms ≥50 μm were inspected in microscope at 10-40x magnification within 6 hours of sampling. Viable organisms were counted and identified based on motility and integrity according to OECD (1985): OECD Test Guideline for Testing of Chemicals 202, "*Daphnia* sp. acute immobilisation test and reproduction test". Viable organisms were identified to Phyla and to species level when possible. Because it can take some hours after treatment to the organism to recover from the reactor unit treatment or to observe the UV irradiation toxicity effect on the organism in the microscope, both treated and control samples at discharge were counted both immediately after discharge sampling and 24h later to ensure the compliance or non-compliance of the results with the D-2 requirement. For discharge samples, the ≥50 μm organism were counted in control and treated samples both immediately after discharge sampling and 24h after discharge sampling as described in chapter 4.3.5. During the 24h storage, the samples were maintained in 50ml glass bottles in the dark and in cooling temperature before re-counting on board or on shore. Because of the high density of organism in the control samples inducing a risk for oxygen lack during storage time, the control samples were diluted before the 24h storage to 1:25 by mixing 2 ml of the control sample in 50 ml of non-concentrated control water to ensure survival conditions in control samples were comparable to those in the treated samples. The 24h after discharge treatment results for all treated samples were corrected by the mean mortality of the organism in all discharge control samples during the 24h storage after discharge sampling. The preservation and storage conditions are described in the chapter 3.

4.3.6 Organisms ≥10-50 μm

CFDA-staining method

The viability of the micro-plankton (>10-50 μm) is determined by observing cells incubated with 5-carboxyfluorescein diacetate acetoxymethyl ester (CFDA-AM) according to Ganassin et al. (2000). Because of the delayed toxicity typical of UV radiation (damage to DNA that does not immediately kill organisms), the samples for CFDA-AM assay were stored at 4°C during 24h after sampling before to be fixed and frozen until analysis by microscopy. A 10 ml sample is incubated for 1 hour with 4 μmol of CFDA-AM. The sample is fixed with formalin and filtered onto black polycarbonate filters (25 mm). The filter is mounted on a glass slide in paraffin oil and frozen. CFDA-AM is hydrolysed only in a living cell. CFDA-AM is a marker for cell membrane integrity and may be measured directly in cells. In principle, the non-fluorescent chemicals CFDA-AM is taken up in the cytosol, where it becomes hydrolysed into fluorescent end products. These end products are trapped inside the cellular compartment and may be observed in an epifluorescence microscope using excitation filter 485 nm and emission filter of 530 nm. In the epifluorescence microscope viable cells are observable as brightly yellow/green coloured cells, while non viable cells are pale green (heterotrophic cells) or pale green with red autofluorescence of the chloroplast (photoautotrophs). Numbers of viable and non viable cells are counted at a magnification of 300-480 times.

Dilution-culture method

The dilution-culture method is used as a complementary method for testing viability of organisms ≥10-50 μm. The method used is based on Throndsen (1978). Briefly, the dilution series is achieved by adding 1 ml sample to 9 ml of algal growth media (20% Z8 seawater media). After gentle but thoroughly mixing, 1 ml of this sample is further diluted with 9 ml of growth medium. In this way, a series of 10x dilution series are made. Number of dilution steps is set according to the expected cell density on the original sample. The test-tubes are sealed/corked and incubated in a room or cabinet with suitable light and temperature conditions. After two to three weeks, the cultures are examined microscopically and the presence of each species in the tubes is noted. When the growth pattern (presence or absence) of each species through the culture series has been determined, the most probable number (MPN) per unit volume can be estimated from tables. Tables are given in Throndsen (1978).

4.3.7 E. Coli

E. coli was quantified according to Norwegian Standard NS 4792 or NS-EN ISO 9308-3 at a temperature of ca. 44 °C and an incubation period of 18-24 hours. Confirmation at a temperature of 44°C and incubation period of 18-24 hours.

4.3.8 Intestinal *Enterococci*

Intestinal *Enterococci* was quantified according to Norwegian Standard NS-EN ISO 7899-2 at a temperature of ca. 37 °C and an incubation period of 44-48 hours. Confirmation at a temperature of 44°C and an incubation period of 2h.

4.3.9 *Vibrio spp.*

Vibrio species were quantified according to the method described by the American Public Health Association (APHA,1995). The total number of *Vibrio* sp., are determined by filtering of a 1-100 ml sample, the filter is placed on TCBS Cholera-medium agar plates (manufacturer: Oxoid), incubated at 37 °C, and the colonies are counted after 24h incubation.

4.3.10 *Vibrio cholerae* (Serotypes O1 and O139)

Colonies of suspected *Vibrio cholerae* identified above are confirmed by growth on nutrient agar without NaCl and oxidase tests, and when necessary by PCR and serotyping with specific antibodies for the outer antigens O1 and O139.

Table 9 Summary of all chemical measurements.

Parameter	Units	Instrument	Reference	Detection limit	Uncertainty			
					Control	#	Average	Std
Salinity	PSU, ‰	Autosal model 8400A	UNESCO (1981)	0.005	Standard seawater IAPSO: 34,99252 PSU	9	34.9934	0.00105
POC	mg C/l	Thermo Flash 2000	APPLICATION LAB REPORTS, Elemental analysis lab, Carlo Erba. January 1987	1.0 µg/mg	Sulfanilamid	84	Estimated 41.84%, measured 41.66%	0.22 %
TSS	mg/l	Sartorius R200 D weight	NS-EN 872 and NS 4733	0.1mg/l	Double analysis natural sample, TSM > 2 mg/l	32	0.9 % difference	11.1%
UV-trans/cm	%	Perkin-Elmer Lambda 40P UV/VIS spectrophotometer	Refbla nr. 1, 1983, og nr. 2, 1984	0.1%	Humus acid solution	26	97.4	4.9%

Table 10 Summary of all biological measurements.

Parameter	Units	Instrument	NIVA method	Reference	Detection limit
Organisms ≥ 50 µm	org./m^3	Dissecting microscope 10-40x magnification	K 9	OECD Test Guideline (1985)	1/m^3
Organisms ≥10-50 µm	cell/ml	Epifluorescence microscope (excitation filter 485 nm; emission filter 530 nm) at 300-480 times magnification	-	Ganassin *et al.* (2000)	1/ml
Organisms ≥10-50 µm	cell/ml	Serial dilution technique	-	Throndsen (1978)	0.2/ml
E. Coli	cfu/100ml	m-Endo Broth MF 274930 (Difco) after concentration by filtration	J2	NS-EN ISO 9308-3	1 cfu/100 ml
Intestinal *Enterococci*	cfu/100 ml	Specific agar, after concentration by filtration 44 °C, , verification on bile-esculinagar as coloured colonies	-	NS-EN ISO 7899-2	1 cfu/100 ml
Vibrio spp.	cfu/100 ml	T.C.B.S. Cholera-medium Agar CM0333 (Oxoid) after concentration by filtration	-	APHA (1995), terminating the method after determining total count of *Vibrio* and prior to specific identification of *Vibrio cholera*	1 cfu/100 ml
Vibrio cholerae (serotypes O1 and O139)	cfu/100 ml	T.C.B.S. Cholera-medium Agar CM0333 (Oxoid) after concentration by filtration, PCR, Serotyping	-	APHA (1995), terminating the method after determining total count of *Vibrio* and prior to specific identification of *Vibrio cholera*	1 cfu/100 ml

5. Results and discussion

5.1 QA/QC prosedures

Quality assurance and quality control have been performed during the testing according to Chapter 5 in the QAPP and according to G8. All activities and collected data during testing of the KBAL system have been logged as summarized in **Table 11**. For each activity a specially designed log in paper and/or electronic format was used, Appendix A-H, and was used for the respective quality assurance evaluation. The appendixes A-F for all test cycles are collected and presented in a separate document. Appendix G and H were presented in the QAPP document.

Table 11. Log protocols for all activities of the project.

Appendix	Description
A	Total project management
B	Collection and preservation of samples
C	Logging of *in situ* measurements
D	Evaluation form for organisms ≥50 µm
E	Evaluation form for organisms ≥10-50 µm
F	Evaluation form for coliforms, Enterococcus group, intestinal *Enterococci*, *Vibrio cholerae* and *Vibrio cholerae* (serotypes O1 and O139).
G	KBAL's operation manual
H	Configuration of the sampling tube

5.2 Operational performance of the KBAL System

A total of 5 test cycles were completed in the period of June 2011 to March 2012. The dates of the cycles and the tanks used for treated and control waters as the total volume of water transfer are given in **Table 12.** For all test cycles, the total water volume transferred during ballasting was the same than the total volume of water transferred during discharge, for both treated and control waters.

Table 12. Test cycles completed with dates and water volume transfers.

Test name	Ballasting date	Deballasting date	Treated tanks	Control tanks	Treated water total volume	Control water total volume
Cycle 1	22.06.2011	23.06.2011	(8p), 3s, 3p	6p, 6s	2000 m^3	2000 m^3
Cycle 2	19.07.2011	23-24.07.2011	(8p), 3s, 3p	8s	2000 m^3	300 m^3
Cycle 3	23.08.2011	24.08.2011	8p, 8s	6p	900 m^3	650 m^3
Cycle 4	24.08.2011	25.08.2011	8p, 8s	6p	900 m^3	900 m^3
Cycle 5	19.03.2012	20.03.2012	8p, 8s	4p, 4s	900 m^3	1700 m^3

A summary of the operational data recorded in appendix C during ballasting and discharge, as the flow rate for control water and the flow rate, pressures and UV-dose for treated water, is given in the **Table 13** and **Table 14**. As specified in chapter 1.3.2.3 of the QAPP, the Sea KBAL system was designed for treatment of 600 m3/h water supplied by both ship's pump and the KBAL booster pump. The pressure before the reactor shall be minimum 4.5 bara (3.5 barg). Pressure after the reactor shall be 0.012 to 0.03 bara (-0.988 to -0,970 barg). The UV unit should give an UV dose of minimum 60 mJ/cm^2 according to land-based testing results (see NIVA's report No.6164-2011).

As shown in **Table 14,** the average flow rate applied by the KBAL system during both ballasting and discharge was approximatvely 600m^3/h for all test cycles, as specified in QAPP, with an average flow rate range for all water transfers of 598-603m^3/h for all test cycles except for test cycle 1 with a slight

higher flow range of 635-663 m3/h in average. The low standard deviations of the flow rate for each test cycles indicate that the flow rate was stable during the whole process of each water transfer.

For all test cycles, the UV reactor was operated in Step 2, i.e. 80% of the maximal intensity of each of the 12 medium pressure lamps of the UV reactor was applied to the water during both ballasting and discharge processes, in order to simulate the same energi input applied during land-based testing. As shown in **Table 14**, the records of the UV-dose measurements by the UV-sensor inside of the UV chamber during UV irradiation of the treated water show a variation in the range of 119-280mJ/cm^2 during ballasting and 100-246mJ/cm^2 during discharge for the three last test cycles. The average UV dose in test cycle 5 was over two times higher than in test cycles 2 and 3 because of influent water quality differences. As indicated in **Table 15**, the UV-T of the seawater for both test cycles 2 and 3, which were performed in the same periode in August 2011, was in a range of 90-93% in influent water and 92-94% in discharge water, while the UV-T in the influent seawater in test cycle 5conducted later on in March 2012 was higher (99% in influent water and 97-99% in discharge water). The higher UV-T quality of the seawater during the test cycle 5 explains the higher UV-dose measured. The UV dose measurements could not be recorded for the two first test cycles because the UV sensor was defect. Neverthelsess it was checked several times during the whole water transfers that all the 12 lamps were switched on both on control panel and on the UV unit itself. The low standard deviations of the UV dose measurements indicate that the UV-dose applied by the UV reactor was relatively stable during the water transfer process for each test cycle. The UV-dose delivered by the UV unit was according to the QAPP specification, i.e. above the minimum dose of 60mJ/cm^2.

The pressures before the reactor should be above 3.5 barg. The records presented in **Table 14** show a variation from 3.55 to 3.94 barg, including both ballasting and discharge treatments. The pressures after the reactor, that should be lower than -0.970 barg, did vary from -0.966 to -1.000 barg, including both ballasting and discharge tretaments. According to the vendor, the lower pressure recorded after the reactor on the test cycle 5, i.e. below the detection limit of the instrument (-1.000barg), was due to the lower temperature of the water during testing (6ºC in test cycle 5 and >15ºC in all other test cycles). This confirms the vacuum point of the water is lower in cold waters than in warm waters. The relatively low standard deviations of the pressures measurements indicate stability of the system during the water transfer for all test cycles.

Table 13 Average values and standard deviation of the flow data for control water during ballasting and discharge, given as average of 3 measurements during ballasting and 3 measurements during discharge, except for Test Cycle 5 where the flow was calculated.

Flow (m^3/h)	**Control influent**		**Control discharge**	
	average	**Stdev**	**Average**	**stdev**
Test Cycle 1	1333	231	1125	295
Test Cycle 2	1132	753	246	25
Test Cycle 3	720	159	387	88
Test Cycle 4	675	177	612	121
Test Cycle 5	1110*	-	940*	-

* Flowmeter reading was not available, so the flow was calculated from water level in tanks and pumping time.

Table 14 Operational data for treated water during ballasting and discharge, given as average of 3 measurements for ballasting and 9 measurements for discharge according to G8.

		Treated influent		Treated discharge	
Parameter	**Unit**	**Average**	**Stdev**	**Average**	**stdev**
Test cycle 1					
Flow	m^3/h	663	3	635	12
UV dose	mJ/cm^2	12 lamps*	-	12 lamps*	-
Pressure before reactor	barg	3.61	0.05	3.55	0.12
Pressure after reactor	Barg	-0.978	0.003	-0.975	0.001
Test cycle 2					
Flow	m^3/h	602	3	601	5
UV dose	mJ/cm^2	12 lamps*	-	12 lamps*	-
Pressure before reactor	barg	3.93	0.06	3.94	0.07
Pressure after reactor	barg	-0.975	0.001	-0.966	0.001
Test cycle 3					
Flow	m^3/h	599	9	598	13
UV dose	mJ/cm^2	119	5	100	9
Pressure before reactor	barg	3.89	0.07	3.92	0.05
Pressure after reactor	barg	-0.971	0.001	-0.971	0.004
Test cycle 4					
Flow	m^3/h	596	8	600	3
UV dose	mJ/cm^2	132	3	117	11
Pressure before reactor	barg	3.92	0.02	3.93	0.04
Pressure after reactor	barg	-0.977	0.003	-0.977	0.002
Test cycle 5					
Flow	m^3/h	602	1	603	6
UV dose	mJ/cm^2	280	2	246	35
Pressure before reactor	barg	3.9	0.0	3.9	0.0
Pressure after reactor	barg	-1.000	0.000	-1.000	0.000

* The UV sensor was not working but all 12 lamps were checked to be switched“on” during the whole test.

5.3 Chemical water quality

The temperature, salinity, pH, POC, DOC and TOC were measured in both uptake and discharge water (control and treated waters) and the results are given in **Table 15** for all test cycles. The results show variations of the uptake water quality in temperature from approximately 6 to 22 ºC, in salinity from 28 to 31 PSU, in particulate organic carbon from 0.2 to 0.7 mg/l, and suspended solids from 2 to 12 mg/l.

Table 15. Temperature, salinity, particulate organic carbon and total suspended solids in uptake and discharge water (control and treated). Nine parallele samples were collected, except from discharge control water where triplicate samples were collected.

		Uptake water						Discharge water											
		Control			Treated			Treated									Control		
	Sample	S1-1	S1-2	S1-3	S2-1	S2-2	S2-3	S3-1	S3-2	S3-3	S3-4	S3-5	S3-6	S3-7	S3-8	S3-9	S4-1	S4-2	S4-3
Parameter	Unit																		
Test cycle 1																			
tank volume	%	11	48	79	19	40	66	81	-	72	56	49	44	25	13	9	81	53	15
Temperature	ºC	14.3	14.6	14.6	14.2	14.6	14.3	13.4	13.5	13.4	13.4	14.1	14.3	14.2	14.4	14.3	13.8	14.3	14.3
UV-T	%	92.3	91.9	93.9	94.0	92.4	92.0	94.9	94.8	94.8	92	94.8	93.7	93.9	94.4	93.9	93.5	91.5	90.3
Salinity	PSU	28.5	28.7	30.1	30.7	28.5	28.4	28.8	28.8	28.8	28.6	28.6	28.6	28.5	28.6	28.7	29.5	29.5	29.6
POC	mg/l	0.229	0.179	0.181	0.225	0.229	0.256	0.141	0.115	0.136	0.103	0.105	0.093	0.129	0.104	0.138	0.208	0.267	0.373
TSS	mg/l	2.8	2.6	1.8	3.0	3.2	2.8	1.4	2.2	1.8	3.8	2.0	2.4	2.2	1.8	2.2	2.0	5.6	5.0
Test cycle 2																			
tank volume	%	3	31	70	22	49	84	-	81	73	57	49	42	34	26	9	79	38	21
Temperature	ºC	18.2	18.0	18.1	17.7	17.8	18.0	17.6	17.7	17.6	17.7	17.7	17.7	17.7	17.7	17.7	16.9	17.4	17.5
UV-T	%	90.2	90.5	89.7	90.8	90.6	90.3	94.4	93.9	94.4	93.7	93.9	94.1	93.2	94.0	93.5	93.2	93.2	93.1
Salinity	PSU	28.7	28.6	28.4	29.8	29.4	29.5	29.6	29.6	29.6	29.6	29.6	29.6	29.7	29.7	29.6	28.3	28.3	28.4
POC	mg/l	0.408	0.367	0.408	0.414	0.367	0.460	0.182	0.143	0.103	0.137	0.112	0.113	0.141	0.148	0.200	0.149	0.169	0.197
TSS	mg/l	10.7	7.0	8.7	5.7	6.0	9.0	44.6*	2.4	2.0	2.4	1.8	1.6	1.8	1.6	3.8	5.7	3.7	3.3

*Probably an analysis failure by omitting washing of the filter for salt.

Table 15 continued on next page

Table 15. To be continued.

		Uptake water						Discharge water											
		Control			Treated			Treated 500 m3/h									Control		
		S1-1	S1-2	S1-3	S2-1	S2-2	S2-3	S3-1	S3-2	S3-3	S3-4	S3-5	S3-6	S3-7	S3-8	S3-9	S4-1	S4-2	S4-3
Test cycle 3																			
tank volume	%	16	55	96	25	57	81	96	82	72	-	54	41	32	21	9	97	61	28
Temperature	ºC	20.0	19.2	19.0	N/A	N/A	N/A	19.2	19.2	19.2	19.2	19.2	19.2	19.2	19.2	19.2	22.1	18.8	N/A
UV-T	%	92.6	89.7	93.0	93.0	93.1	93.2	93.4	93.5	93.0	93.6	93.5	93.3	93.2	92.6	93.7	93.2	92.7	92.1
Salinity	PSU	30.9	30.5	30.3	31.0	30.9	30.6	30.8	30.7	30.8	30.8	30.8	30.8	30.7	30.8	30.8	30.1	30.2	30.1
POC	mg/l	0.472	0.727	0.433	0.443	0.500	0.525	0.381	0.375	0.378	0.355	0.303	0.389	0.402	0.518	0.298	0.422	0.458	0.446
TSS	mg/l	4.2	11.8	3.8	3.2	2.0	2.8	7.0	4.0	3.8	4.0	4.6	4.2	5.2	4.2	3.8	1.8	3.6	5.0
Test cycle 4																			
tank volume	%	20	67	78	66	66	83	97	86	77	64	57	49	40	31	21	60	42	18
Temperature	ºC	19.1	19.2	N/A	19.1	18.7	18.9	19.2	19.2	19.2	19.2	19.2	19.2	19.2	19.2	19.2	19.0	19.0	19.1
UV-T	%	90.9	91.3	91.6	93.6	93.0	92.0	94.2	91.7	93.9	94.2	93.5	93.3	93.9	92.4	93.6	93.3	93.0	93.3
Salinity	PSU	30.1	29.8	29.5	30.4	30.3	30.3	30.2	30.2	30.2	30.1	30.1	30.2	30.1	30.2	30.1	29.8	29.8	29.9
POC	mg/l	0.489	0.341	0.346	0.466	0.517	0.678	0.230	0.288	0.294	0.316	0.247	0.319	0.302	0.335	0.343	0.356	0.416	0.473
TSS	mg/l	3.0	1.6	3.4	2.6	3.6	2.8	2.6	4.6	3.2	3.2	2.0	2.8	1.8	2.8	3.2	1.0	2.2	4.0
Test cycle 5																			
tank volume	%	21	55	93	10	53	86	89	81	71	43	36	29	26	N/A	20	91	51	42
Temperature	ºC	6.4	6.4	6.8	6.5	6.4	6.5	7.0	7.0	7.1	7.0	7.1	7.1	7.1	N/A	N/A	N/A	N/A	N/A
UV-T	%	98.9	98.9	99.2	99.3	99.5	99.0	97.3	98.8	99.1	99.2	98.5	98.8	98.6	98.3	97.7	99.0	98.9	97.0
Salinity	PSU	31.6	31.6	31.6	30.7	31.8	31.6	31.7	31.6	31.5	31.3	31.3	31.3	31.1	31.1	31.2	31.2	31.3	31.3
POC	mg/l	0.392	0.358	0.275	0.305	0.208	0.325	0.201	0.218	0.190	0.328	0.396	0.443	0.395	0.453	0.549	0.308	0.290	0.558
TSS	mg/l	6.4	4.6	5.0	4.0	4.2	3.6	5.8	6.8	5.5	9.0	9.5	14.8	11.5	12.8	16.8	5.5	5.3	17.5

5.4 Fulfillment of the biological water quality criteria and biocidal effects on organisms ≥50 µm in minimum diameter

The number of viable organisms ≥50 µm in minimum diameter, as determined on the basis of motility and integrity by microscope examination, on uptake and discharge of water for treated and control ballast water is given in **Table 16.** For a test to be valid, both the control uptake water and the ballast water to be treated must contain organism concentration 10 times the value of regulation D-2.1 and control water viable organism concentration upon discharge exceeding the values of Regulation D-2.1. For the performance of the ballast water treatment system to pass the regulation D-2 of the IMO Guidelines, as given in **Table 2**, less than 10 viable organisms ≥50 µm in minimum diameter per m^3 should be present.

Results shown in **Table 16** are given as the mean value of 3 samples for both uptake waters and discharge of control water, while discharge of treated water is the mean of 9 samples (except for test cycle 3 where only 8 samples could be taken during the discharge process). Because it can take some time before the effect of UV-treatment is observable as death in the organism in the microscope, both treated and control samples at discharge were re-counted 24h later to ensure that the effect was manifested. In order to take in account the mortality due to the 24h storage sampling procedure and not to the KBAL treatment, the 24h results for treated samples were corrected with the mortality observed in one control sample stored for 24h (test cycles 1, 3 and 4) and with the mean mortality observed in all three control samples stored for 24h in test cycle 5. The storage conditions are described in the chapter 4.3.5. For treated water, the averages of the 24h re-counting results of all discharge samples are given both before and after correction with the control samples mortality. Recounts of control samples were performed on those groups which had survived in the treated samples. Therefore, the mortality percentage can not be derived from the densities in **Table 16**.

The minimum density criteria of 100 organism/m^3 for uptake water was fulfilled for all test cycles, both for treated and control waters (**Table 16**) with a density in the range from 5 000 to 180 000 organism/m^3. The minimum density criteria of >10 organism/m^3 in control water after discharge was fulfilled for all test cycles, with density ranging from 3 000 to 63 000 organisms/m^3.

Less than 10 organisms/m^3 in treated water immediately after discharge was only fulfilled in test cycle 5, with 0.6 organism/m^3, where the pressure difference before and after the reactor was the highest of all test cycles with pressure after the reactor measured below the sensor detection limit of -1.000barg. For the rest of the test cycles, the average of the densities immediately after discharge ranged from 10 to 77 organisms/m^3. The high densities observed for test cycles 1 and 2, which were 27 and 77 organism/m^3, respectively, might be explained by a contamination by untreated water during discharge. This is based on observation of viable species that were not observed in the influent water. It was suspected that water from a ballast tank filled with untreated water leaked into treated water during discharge. For test cycles 3-5, a more stringent preparation procedure was enforced where pipelines and ballast tanks were flushed several times with treated water before testing and only the ballast water tanks no. 8P and 8S were used. After this procedure correction, the density of organism immediately after discharge was in the range from 0.6 to 12.8 organisms/m^3.

After re-counting of the discharge treated water 24h after sampling, the three last consecutive test cycles, i.e. test cycles 3, 4 and 5, fulfilled the G8 requirement, both before and after correction for organism mortality observed in control samples. The results for these test cycles ranged from 0.3 to 1.2 before correction and from 0.6 to 2.6 organisms/m^3 after correction with mortality observed in control samples.

Table16. Average values and standard deviation of viable organisms ≥50 µm in minimum diameter in uptake (treated and control) and discharge (treated and control) ballast water. Green background indicates that required level was fulfilled, while red background indicates failure to fulfil required level.

	Uptake water		Discharge water					
			Treated			Control		
	Treated	Control	0h	24h	24h corrected	0h	24h	24h mortality
Organisms ≥50 µm in minimum diameter (individuals/m^3)								
Requirement	>100	>100	<10	<10	<10	>10	>10	%
Test cycle 1	43 278 ± 4 945	27 359 ± 6 751	27 ± 32	9.8* ± 17.7	23	18 421 ± 377	8 883 **	51 %
Test cycle 2	5 505 ± 670	5 120 ± 588	77 ± 23	7.3*** ± 4.2	-	4 382 ± 1 270	***	-
Test cycle 3	181 625 ± 8 205	172 773 ± 94 788	12.8**** ± 9.8	1.1 ± 1.1	2.6	203 338 ± 96 566	63 300 **	57 %
Test cycle 4	147 813 ± 29030	128 833 ± 7072	10.7 ± 12.1	1.2 ± 2.4	2.4	100 033 ± 12266	46 375 **	51 %
Test cycle 5	22 569 ± 5 181	22 822 ± 5 642	0.6 ± 1.3	0.3 ± 1.0	0.6	8 834 ± 5 391	3 177 ± 1709	49 %

* Lack of oxygen in some of the samples because of high organic content in the samples might have increased the mortality in some of the samples during the 24 h storage.
** Only one of the three control sample was re-counted after 24h storage for test cycles 1, 3 and 4.
*** The treated water samples were re-counted after 12-17 hours after sampling. There was no control of the mortality in the control samples as the control water was discharged 24h after the treated water.
**** Only 8 samples for treated water could be collected during the sampling of the whole discharge because the sample S3-2 was accidently lost during the rincing of the plankton net.

5.5 Fulfillment of the biological water quality criteria and biocidal effects on organisms ≥10-50 µm in minimum diameter

The number of viable organisms ≥10-50 µm in minimum diameter, as determined by the serial dilution method in algal growth medium and by microscopy examination after incubation with CFDA-AM, on uptake (treated and control) and discharge (treated and control) ballast water is given in **Table 17**. For a test to be valid, both the control uptake water and the ballast water to be treated must contain organism concentration 10 times the value of regulation D-2.1, which ha been interpreted by IMO to be >90 cells/ml, and control water viable organism concentration upon discharge exceeding the values of Regulation D-2.1 and therefore >10 cells/ml. For the performance of the ballast water treatment system to pass the regulation D-2 of the IMO Guidelines, as given in **Table 2**, less than 10 viable organisms ≥10-50 µm in minimum diameter per ml should be present.

The minimum density criteria in uptake waters for ≥10-50 µm organism of >90 cells/ml was fulfilled only for the last three consecutive test cycles, i.e. test cycles 3, 4 and 5. For both influent treated water

and influent control water, the average of organism density was in the range from 256 to 508 cell/ml with CFDA analysis method that gives better accuracy for high cell densities. Therefore test cycles 1 and 2 were invalid, while the last three test cycles were both valid and successful, with the criteria for treated water after discharge of <10 cells/ml being fulfilled in all three last test cycles. The organism density averages in treated water after discharge were below the detection limit for all test cycles, except for test cycle 5 with 0.9 cell/ml in dilution culture and 3.2 cells/ml with CFDA method. Even if the dilution culture method will in most cases give a minimum estimate of viable cells as not all species will grow in the growth media used, it was observed similar results with both dilution culture method and CFDA methods in all treated water samples.

The criteria for control water after discharge of >10 cell/ml was fulfilled for all succesfull test cycles.

Table 17. Average values of viable organisms ≥10-50 µm in minimum diameter in uptake and discharge treated and control ballast water. For dilution method, 95% confidence intervals are given. For microscope counts, standard deviations are given. Green background indicates that required level was fulfilled, while red background indicates failure to fulfil required level.

	Uptake water		**Discharge water**	
	Treated	**Control**	**Treated**	**Control**
Organisms ≥10-50 µm in minimum diameter (individuals/ml)				
Requirement	>90	>90	<10	>10
Dilution-culture method **95 % confidence interval**				
Test cycle 1	**14**	**9**	**<0.3**	**20**
(95 % conf. int.)	4-41	3-39	<0.1-1.7	10-140
Test cycle 2	**19**	**15**	**<0.3**	**10**
(95 % conf. int.)	10-140	5-51	<0.1-1.7	3-40
Test cycle 3	**618**	**630**	**<0.3**	**440**
(95 % conf. int.)	200-2800	200-2800	<0.1-1.7	200-2100
Test cycle 4	**330**	**470**	**<0.3**	**140**
(95 % conf. int.)	150-1300	200-2400	<0.1-1.7	50-510
Test cycle 5	**333**	**1171**	**0.9**	**357**
(95 % conf. int.)	200-2400	300-4800	0.8-6.3	200-2400
CFDA Microscope counts				
Test cycle 1	66 ± 16	53 ± 10	<1	43 ± 9
Test cycle 2	76± 6	71 ± 14	<1	35 ± 8
Test cycle 3	311 ± 118	508 ± 32	<1	520 ± 100
Test cycle 4	508 ± 230	256 ± 36	<1	475 ± 120
Test cycle 5	259 ± 107	416 ± 156	3.2 ± 2.7	431 ± 204

5.6 Biocidal effect on organisms <10 µm in minimum diameter

In addition to the IMO requirements for ≥50µm organism and 10-50µm organism, the <10µm organism were also analysed in all samples with either the dilution culture method or the CFDA method. The results are presented in the **Table 18** only as indicative information.

The results show that the KBAL system had also an effective biocidal effect on organism <10µm. Hence, with an average density in uptake water ranging from 200 to 2000 cells/ml, the average density after discharge was below 2 cells/ml in all treated water samples analysed. The average density of the organism was above 10 cells/ml in all control samples after discharge, ranging from 100 to 900 cells/ml.

Table 18. Summary of results for viable organisms <10µm in minimum diameter in uptake and discharge treated and control ballast water by microscope counts with standard deviation and/or by dilution culture with 95% confidence interval. The results are given as supplementary information because there are no G8 requirement for this organism size group.

	Uptake water		**Discharge water**	
	Treated	**Control**	**Treated**	**Control**
Organisms <10 µm in minimum diameter (individuals/ml)				
None G8 Requirement	-	-	-	-
Dilution-culture method (95% confidence interval)				
Test cycle 1	2000 (100-14 000)	1400 (400-4 100)	2 (0.1-10)	967 (300-3 500)
Test cycle 2	na	na	na	na
Test cycle 3	200 (100-1400)	200 (100-1400)	<0.3 (<0.1-1.7)	200 (100-1400)
Test cycle 4	500 (200-2400)	200 (100-1400)	<0.3 (<0.1-1.7)	300 (150-1300)
Test cycle 5	173 (100-1400)	296 (100-1400)	0.2 0.1-1.7	106 (30-480)
CFDA Microscope counts				
Test cycle 1	na	na	na	na
Test cycle 2	483 ± 25	427 ± 70	<1	223 ± 38
Test cycle 3	na	na	1.3 ± 1.5	na
Test cycle 4	na	na	<1	na
Test cycle 5	na	na	2.3 ± 2.5	na

na: not analysed.

5.7 Bactericidal effects

The numbers of thermotolerant coliform bacteria, *Vibrio* sp., *Vibrio cholerae* and intestinal *Enterococci*, determined as described in section 2.6.4, on uptake and discharge of water for treated and control ballast water is given in **Table 19**. Regulation D-2 only requires discharge standards to be met on *Escherichia coli* (a thermotolerant coliform bacteria), *Vibrio cholerae* (toxicogenic serotypes O1

and O139) and *Enterococci* given as a maximum allowable residual concentration at disharge; <250 cfu/100ml, <1 cfu/100ml and <100 cfu/100ml, respectively.

The results in Table 19 show bacteriological density in uptake water for both treated and control waters, ranging from 1 to 20 cfu/ml for thermotolerant coliforms, and from 3 to 82 cfu/ml for vibrio species. Enterococcus species were not detected. In treated water samples after discharge, neither *E.coli*, *Vibrio cholerae* nor *intestinal enterococci* were detected. Therefore, all treated water samples after discharge fulfilled the D-2 requirements.

Table 19. Culturable thermotolerant coliform bacteria, *Vibrio* sp. and intestinal *Enterococci* in uptake and discharge treated and control ballast water. Green background indicates that required level was fulfilled, while red background indicates failure to fulfil required level.

	Uptake water		**Discharge water**	
	Control	**Treated**	**Control**	**Treated**
Thermotolerant coliform bacteria (and *Escherichia coli in brackets) (cfu/100 ml)**				
Requirement	-	-	-	<250*
Test cycle 1	2.3 ± 2.5	1.3 ± 1.5	15 ± 3	<1
Test cycle 2	14 ± 2	19 ± 9	<1	<1
Test cycle 3	<1	<1	2.3 ± 1.2	<1
Test cycle 4	13 ± 9	<1	6 ± 3	<1
Test cycle 5	5 ± 9	0.7 ± 0.6	1.3 ± 0.6	<1
Vibrio* sp. (and *Vibrio cholerae in brackets) (cfu/100 ml)**				
Requirement	-	-	-	(<1**)
Test cycle 1	11 ± 6	9 ± 1	>200	<1
Test cycle 2	63 ± 25	57 ± 21	193 ± 133	<1
Test cycle 3	29 ± 32	3 ± 2	191 ± 55	3 ± 2 (<1)
Test cycle 4	82 ± 102	14 ± 4	>200	6 ± 10 (<1)
Test cycle 5	53 ± 34	14 ± 6	1 ± 2	<1
Enterococcus group (and Intestinal *Enterococci** in brackets) (cfu/100 ml)**				
Requirement	-	-	-	<100***
Test cycle 1	<1	<1	9 ± 6	<1
Test cycle 2	<1	<1	<1	<1
Test cycle 3	<1	<1	<1	<1
Test cycle 4	<1	<1	<1	<1
Test cycle 5	<1	<1	1 ± 1	<1

* The figures refer to the number identified as *Escherichia coli*/100 ml within the group of thermotolerant coliform bacteria. There is a requirement for *Escherichia coli* being <250 cfu/100ml in discharge treated water (Regulation D-2).
** The figures refer to the number of *Vibrio cholerae*/100 ml. There is a requirement for toxicogenic *Vibrio cholerae* (serotypes O1 and O139) being <1 cfu/100 ml in discharge treated water (Regulation D-2).
*** The figures refer to the number identified as intestinal *Enterococci*/100 ml within the group of Enterococcus. There is a requirement for intestinal *Enterococci* being <100 cfu/100ml in discharge treated water (Regulation D-2).

6. Conclusion

The Sea KBAL ballast water management system of Knutsen Shipping AS was developed by Knutsen Shipping AS and shipboard-tested by NIVA. The KBAL treatment system applies pressure drop to remove or inactivate larger organism followed by UV irradiation to remove or inactivate smaller organisms. In total, five shipboard test cycles were completed in the period from June 2011 to March 2012 onboard the m/t *Gijon Knutsen* in the area of Skagen in Denmark and Rotterdam in Holland. All the results are reported in this document. Overall, the three last consecutive test cycles fulfilled the G8 requirements both for uptake water, treated water and control water after discharge. These 3 test cycles were considered as both valid and successful according to G8 requirements from IMO. Recorded operational data for flow rate, water pressures before and after the reactor unit, and UV doses were in accordance with the QAPP specifications and specifications for operation of the KBAL system.